ANTIMICROBIAL ACTIVITY OF SOME MEDICINAL PLANT EXTRACTS AGAINST BACTERIA CAUSING DIARRHOEA

by

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DECLARATION

I declare that **ANTIMICROBIAL ACTIVITY OF SOME MEDICINAL PLANT EXTRACTS AGAINST BACTERIA CAUSING DIARRHOEA** is my own work and that all sources that I have used or quoted have been indicated and acknowledged by means of complete references.

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DEDICATION

This dissertation is dedicated to my almighty father Jehovah God, through whom I had the strength and vigour to start and complete this work.

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ABSTRACT

Infectious diarrhoea is the second largest single cause of mortality in children under the age of five globally. Bacteria are responsible for most diarrhoeal episodes especially in developing countries, and progressive increase in antimicrobial resistance has given rise to the need to investigate other sources of therapy such as medicinal plants. Ten plant extracts were analysed for their antimicrobial activities using the agar well diffusion and broth microdilution method. Their phytochemical contents were screened, and their effect on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was used to assess their antioxidant activities. Their toxicity profiles were evaluated using the XTT Cytotoxicity Assay. Water and methanol extracts of *Adansonia digitata* seeds and pulp showed no inhibition against all the test organisms, while water and methanol extracts of *A. digitata* leaves showed inhibition, with minimum inhibitory concentration (MIC) ranging from 0.39 to 6.25mg/ml. Water and methanol extracts of *Garcinia livingstonei* and *Sclerocarya birrea* barks showed good activity against all the test organisms, with MICs between 0.39 and 1.56 mg/ml. Alkaloids, phenols, flavonoids, saponins, tannins, and terpenoids were found in one or more of the plant extracts, and all the plant extracts demonstrated scavenging power against DPPH.The cytotoxicity of extracts of *Garcinia livingstonei*, and *Sclerocarya birrea* barks ranged between 105.9 µg/ml and 769.9 µg/ml. The results obtained in this study validate the traditional use of *A. digitata* leaves, *G. livingstonei* and *S. birrea* bark in treating bacteria causing diarrhoea.

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic acid
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl Sulphoxide
DPPH	1,1-diphenyl-2-picrylhydrazyl
EC_{50}	Half Maximal Effective Concentration
ELISA	Enzyme-linked Immunosorbent Assay
HEK	Human Embryonic Kidney
HCL	Hydrochloric Acid
HPLC	High Performance Liquid Chromatography
IC_{50}	50% Inhibitory Concentration
MS	Mass Spectrometry
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
Mm	Millimetres
NaOH	Sodium Hydroxide
PS	Penicillin/Streptomycin

Rf	Retention Factor
TLC	Thin Layer Chromatography
XTT	(2,3-Bis-(2-methoxy-4-Nitro-5-Sulfophenyl)-2*H*-Tetrazolium-5-carboxanilide)

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Diarrhoea and pneumonia together account for up to 29% of all child deaths globally, with children living in remote communities being affected most often (WHO 2013). Infectious diarrhoea is a major public health concern worldwide. It is still the second largest single cause of mortality in children under the age of five globally, and the largest cause in Sub-Saharan Africa (UNICEF 2010). Diarrhoea continues to kill more children under the age of five years than AIDS, malaria and measles combined (Black et al. 2010). In 2010, an estimated 1•731 billion episodes of diarrhoea (36 million of which progressed to severe episodes) occurred in children younger than five years and in 2011, an estimated 700 000 episodes of diarrhoea led to death. Most of the deaths occurred within the first two years of life with diarrhoea accounting for 72% of these deaths (Walker et al. 2013).

In South Africa, of the 72 553 deaths recorded in children under the age of five in 2008, deaths caused by diarrhoea amounted to 6 293, making diarrhoea the third largest cause of death in children in this age group (Black et al. 2010).

In developing countries, diarrhoea often results from food and water contaminated by *Salmonella typhi*, *Campylobacter jejuni* and Shiga toxin-producing *Escherichia coli* (STEC), and water sources contaminated by *Giardia intestinalis* and *Cryptosporidium parvum* (Mathabe et al. 2006; Aboutalebet al. 2014). *Shigella spp*., *Salmonella spp*., *Campylobacter jejuni* and the protozoan *Entamoeba histolytica* are notably responsible for acute bloody diarrhoea (Farthing and Kelly 2007).

Besides bacteria, infectious diarrhoea can be caused by viruses and parasites. The prevalence of each pathogen varies with geographical region and population factors. Bacteria, for instance, are responsible for 10–55% of diarrhoeal episodes, with the highest number of episodes occurring in the developing world (Fhogartaigh and Edgeworth2009). This is because sanitation, hygiene and safe drinking water are directly related to economic development, and over time this has effectively defined the incidence and prevalence of many of the bacterial agents of enteric infections. For example, cholera, shigellosis and typhoid are most common in the most underserved populations, with greater incidence at times of limited water supply and flooding, during which water supplies can be contaminated by sewage (Petri et al. 2008). Viruses on the other hand, are more common in infants and children, particularly in developed countries because some viruses are geographically ubiquitous, such as rotavirus, which is estimated to infect 90% of the population of the world younger than five years of age (Petri et al. 2008; Odiit et al. 2014).

Microbial infections followed by inflammation and oxidative stress are also associated with gastrointestinal tract disorders (Ahmedet al. 2013). Reactive metabolites of oxygen and nitrogen through oxidation and free radical reactions are believed to be responsible for inflammatory bowel disease and contribute to causing diarrhoea by acting as secretagogues (Gaginella et al. 1995).

Escherichia coli has been implicated as one of the major bacteria causing diarrhoea. In an outbreak of haemolytic uraemic syndrome and bloody diarrhoea caused by a virulent *Escherichia coli* strain O104:H4 in Germany (with some cases elsewhere in Europe and North America), 810 cases of the syndrome and 39 deaths occurred in May, 2011 (Bielaszewska et al. 2011).

As of June 2011, 15 cases of haemolytic uraemic syndrome or bloody diarrhoea was also identified in Gironde, south-west France. Investigations suggest that the vehicle of transmission was sprouts, served at an event in Bègles on 8 June 2011. A strain of shiga toxin- producing *Escherichia coli* O104:H4 was also isolated from five of the cases presented (Gault et al. 2011).

An outbreak of *Shiga bacillus* (*Shigella dysenteriae* type 1) infection was detected for the first time in KwaZulu-Natal, South Africa in 1994. Forty-eight cases of this epidemic that presented at a referral hospital were clinically evaluated and patients of all age groups presented with dysentery. The isolates were demonstrated to be pathogenic by *in vitro* testing for invasion and toxin production, and were found to be resistant to first-line antibiotics used for the treatment of shigellosis, namely ampicillin, cotrimoxazole, tetracycline and chloramphenicol. However, they were susceptible to nalidixic acid, ceftriaxone and ciprofloxacin (Pillay et al. 1997).

1.2 PROBLEM STATEMENT

In South Africa, diarrhoeal disease accounts for 24% of deaths among children aged one to five years (Statistics South Africa 2008). Diarrhoea is also a leading cause of morbidity and mortality in HIV-infected children. HIV-infected children admitted with diarrhoea are more likely to have prolonged diarrhoea and malnutrition and require a longer hospital stay. They also have a higher frequency of recurrent diarrhoea and recurrent hospital admissions (Chhagan and Kauchali 2006).

A major consequence of diarrhoea in young children is the need for medical and parental care, clinic visits, hospitalisation, and loss of work by the parents or the caregiver. In a study carried out to estimate the costs associated with diarrhoeal

disease of all aetiologies in children less than five years of age at Dr George Mukhari Hospital, a tertiary level hospital in Gauteng, South Africa, the mean duration of hospital stay was between 4.6 and 5.7 days, while the mean total in-patient cost was between R5963 and R7256. This shows the significant burden of diarrhoea on the South African health system (MacIntyre et al. 2010).

A joint statement by the World Health Organization and United Nations Children's Fund in 2004 recommended the use of low osmolarity oral rehydration solution along with zinc for 14 days as an adjunct therapy to decrease diarrhoeal deaths among the world's most vulnerable children (Fischer et al. 2009). Antibiotics however are to be used only for acute bloody diarrhoea (stools with visible blood) (Bhatnagar et al. 2004). The drug of choice is co-trimoxazole if local prevalence of resistance in *Shigella* is less than 30%; nalidixic acid if resistance exceeds 30%, norfloxacin, ciprofloxacin or a third-generation cephalosporin must be used as second and third line drugs (Deepali et al. 2011).

The progressive increase in antimicrobial resistance among enteric pathogens in developing countries is becoming a critical area of concern. Treatment of diarrhoea caused by bacteria with antibiotics often results in drug resistance in both enteric and non-enteric diarrhoea-causing bacteria (Fhogartaigh and Edgeworth2009). This is most likely related to the frequent unrestricted use of over-the-counter drugs without medical supervision.

Antibiotic resistance to trimethoprim-sulfamethoxazole, ampicillin, chloramphenicol, and tetracycline was noted among *Campylobacter* spp, *Shigella*, *Salmonella* and *E. coli (*Isenbarger et al. 2002). High resistance to antimicrobials, such as erythromycin, ciprofloxacin, vancomycin, and fusidic acid by *Campylobacter* spp. isolated from human diarrhoeal stools was demonstrated in Vhembe district, South Africa (Samie

et al. 2007). Samie et al. (2012) demonstrated that unsafe drinking water was the cause of diarrhoea among HIV-infected patients in Makhado municipality of the Limpopo province of South Africa. *Acinetobacter lwoffii*, *Vibro fluvialis*, *Enterobacter cloacae*, *Shigella* spp., *Pseudomonas* spp. and *Yersinia enterocolitica* were isolated from water samples taken and were found to be highly resistant to cefazolin (83.5%), cefoxitin (69.2%), ampicillin (66.4%), and cefuroxime (66.2%). Intermediate resistance was observed against gentamicin (10.6%), cefepime (13.4%), ceftriaxone (27.6%), and cefotaxime (29.9%).

Antibiotics such as amoxicillin/clavulanate and co-trimoxazole, sometimes used in the treatment of infectious diarrhoea of bacterial origin, may paradoxically cause hepatotoxic reactions. Other side effects of antibiotics include cutaneous reactions, gastrointestinal disorders and yeast overgrowth (Andrade et al. 2011).

At present, the continued development of new antimicrobials, other than antibiotic ones, particularly those used for the treatment of diarrhoea in children, is critically important.

1.3 PURPOSE OF THIS RESEARCH

The purpose of this study was to investigate the use of selected plant extracts, *Adansonia digitata*, *Garcinia livingstonei*, and *Sclerocarya birrea,* in the treatment of diarrhoea caused by some bacteria. This may lead to the discovery of an alternative form of treatment other than antibiotics being used at present, to which many of the bacteria are developing resistance. Many plants have been used traditionally in the treatment of diarrhoeal diseases and researchers have noted that further investigations on these plants might lead to the development of antimicrobial drugs

of natural origin that may combat the rapid development of multiple resistances to the available antibiotics by pathogens.

1.4 LAYOUT OF THE DISSERTATION

This dissertation has five chapters, which are arranged as follows:

Chapter 1: Introduction

The introduction to the study provides an overview and background to the study. This section also outlines the problem statement, purpose of the study and explains the layout of the dissertation.

Chapter 2: Literature review

The literature review in chapter 2 provides an overview of existing literature on medicinal plant extracts and their antimicrobial activities against diarrhoea-causing bacteria.

Chapter 3 and 4: Research

These chapters outline the research outputs emanating from the different research objectives that have been submitted for publication to peer-reviewed journals.

Chapter 5: General discussion and conclusions

This chapter discusses the results of all the experiments in relation to one another. Conclusions of the study are provided and recommendations for improvements are made.

CHAPTER 2: LITERATURE REVIEW

2.1 ANTIMICROBIALS

In an attempt to combat the various forms of disease that have continued to plague humans from time immemorial to this day, different types of antimicrobials have been developed to fight the pathogens responsible for these diseases. Antimicrobials, which are substances that kill or inhibit the growth of microorganisms, could be in the form of antibiotics, which are products of microorganisms or synthesised derivatives (Cowan 1999), antimicrobial peptides produced by complex organisms as well as some microbes (Jenssen et al. 2006) and medicinal plants, which appear to be the focus of mainstream medicine today (Cowan 1999).

2.1.1 TYPES AND SOURCES OF ANTIMICROBIALS

Different types of antimicrobials exist: antibiotics, anti-viral, anti-fungal, anti-protozoan etc. Antibiotics are used in the treatment of bacterial infections and can be obtained from either natural or synthetic sources. Examples of those with a natural origin are phenyl propanoids (chloramphenicol), polyketides (tetracycline), aminoglycosides (streptomycin, gentamycin), macrolides (erythromycin), glycopeptides (vancomycin) and second-generation β-lactams (cephalosporins). Those from synthetic sources are sulphonamides, quinolones and oxazolidinones. Most antibiotics exert their action either by inhibition of the bacterial cell wall or protein synthesis. Exceptions are the quinolones that inhibit DNA synthesis, and the sulphonamides that inhibit the synthesis of metabolites used for the synthesis of deoxyribonucleic acid (DNA) (Singh and Barrett 2006). Most anti-viral, anti-fungal, anti-protozoa and anti-cancer drugs however are obtained from synthetic sources.

Because of the re-occurring resistance of pathogenic microorganisms to antibiotics, as well as the side effects presented by these antibiotics, investigation of other sources of antimicrobials, such as medicinal plants, for their antimicrobial properties is gaining ground. Plants produce secondary metabolites (phytochemicals), which have demonstrated their potential as antibacterials when used alone and as synergists or potentiators of other antibacterial agents. Phytochemicals frequently act through different mechanisms than conventional antibiotics and could therefore be of use in the treatment of resistant bacteria (Abreu et al. 2012).

2.2 MEDICINAL PLANTS

2.2.1 HISTORY OF MEDICINAL PLANTS

Human use of plants as medicines could be dated back to the Middle Paleolithic Age, which is about 60 000 years ago, according to fossil records (Fabricant and Farnsworth 2001). The first records written on clay tablets in cuneiform are from Mesopotamia and date from about 2 600 BC. Some of the substances that were used were oils of *Cedrus* species (cedar) and *Cupressus sempervirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrrh) and *Papaver somniferum* (poppy juice), most of which are still in use today for treating ailments ranging from coughs and colds to parasitic infections and inflammation (Gurib-Fakim 2006).

Health care in ancient times included the use of leaves, flowers, stems, berries and roots of herbs for their therapeutic or medicinal value. These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations (Balick and Cox, 1996; Samuelsson 2004). Knowledge of the specific plants to be used and the methods of application for particular ailments were passed

down through oral history and information regarding medicinal plants was eventually recorded in herbals (Balunasa and Kinghorn 2005).

2.2.2 GENERAL USES OF MEDICINAL PLANTS

Medicinal plants (otherwise referred to as herbs, herbal medicines, pharmacologically active plants or phytomedicinals) remain the dominant form of medicine in most countries. Over three fourth of the earth's population depend primarily on raw plant products to meet their daily health care needs (Barrett and Kieffer 2001). Most of the plant materials collected are used fresh in order to obtain the extract from the whole plant or parts of it, which could be leaves, roots, flowers or fruit. In case of woody forms, mostly the bark, roots and other parts are used. Carminatives such as ginger, cloves and coriander are also usually added as fresh or dried materials (Rao and Arora 2004). For example, bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) have been reported in different manuals of phytotherapy to treat urinary tract infections while species such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tea tree (*Melaleuca alternifolia*) are described as broad-spectrum antimicrobial agents (Heinrich et al. 2004).

In South Africa and Uganda, many individuals in the rural areas receive their health education and health care from practitioners of traditional medicine (Jager et al. 1996; Light et al. 2005; Lewis, 2014). In the Eastern Cape Province, of South Africa, *Elephantorrhiza elephantina*, *Hermannia incana*, *Pelargonium reniforme*, *Alepidea amatymbica* and *Bulbine latifolia* are the plants recommended most frequently for the treatment of diarrhoea by both traditional healers and rural dwellers. Roots, bark and leaves are the common parts of plants used, while decoctions and infusions are the main methods of preparation (Appidi et al. 2008).

Some plant extracts with great medicinal value are the stem bark decoction of *Albizia gummifera,* which is used in the management of venereal diseases (Buwa and Van Staden 2006), leaves of *Glyphaea brevis*, which are macerated in water and are used to treat intestinal diseases and hepatitis (Noumi and Yomi 2001) and oil extracts of the roots, seeds and stem barks of *Monodora myristica,* which are used to cure scabies, helminthiasis, malaria and dysenteric syndromes (Okpekon et al. 2004).

Many plants used as traditional medicines are now being validated through scientific research by isolation of bioactive compounds for direct use in medicines. For instance, drug discovery from medicinal plants led to the isolation of early drugs such as morphine from opium, cocaine, codeine, digitoxin and quinine, some of which are still in use (Balunasa and Kinghorn 2005; Samuelsson 2004).

More recently a drug, β-methoxypsoralen, has been produced from the plant *Ammi majus* (bishop's weeds), which was reported by Egyptian medical practitioners to treat vitiligo, a skin condition characterised by the loss of pigments. This drug is now used to treat psoriasis and other skin disorders, as well as T-cell lymphoma (Gurib-Fakim 2006).

2.3 ACTIVE COMPONENTS OF PLANT EXTRACTS

The beneficial medicinal effects of plant materials typically result from the combination of secondary products present in plants. These compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are synthesised and deposited in specific parts or in all parts of the plant (Joseph and Raj 2010). Generally, leaves are the favourable storage site for desired

compounds. Fruits also contain a substantial amount of active ingredients, and thus are often consumed as juice via oral administration to obtain the desired compounds. Other parts of plants that can be extracted for therapeutic compounds are roots, aerial parts, flowers, seeds, stem barks, etc. (Chan et al. 2012).

Plant secondary metabolites are used as the basis for the production of valuable synthetic compounds such as pharmaceuticals, cosmetics, or more recently nutraceuticals (Bourgaud et al. 2001). These secondary metabolites are largely viewed as potential sources of new drugs, antibiotics, insecticides and herbicides (Crozieret al. 2006). This is because of their biological significance and potential health effects, such as antioxidant, anticancer, anti-aging, anti-atherosclerotic, antimicrobial and anti-inflammatory activities.

2.4 MECHANISM OF ACTION OF PLANT SECONDARY COMPOUNDS

Plant secondary compounds are usually classified according to their biosynthetic pathways. Three large molecular families are generally considered: phenolics, terpenes and steroids, and alkaloids. A good example of a widespread metabolite family is the phenolics, because these molecules are involved in lignin synthesis, they are common to all higher plants. Phenolic compounds are potent antioxidants and free radical scavengers which can act as hydrogen donors, reducing agents, metal chelators and singlet oxygen quenchers (Chew et al. 2009). Studies have shown that phenolic compounds such as catechin and quercetin are very efficient in stabilising phospholipid bilayers against peroxidation induced by reactive oxygen species (Gülçin et al. 2010; Gülçin 2010). Flavonoids, which are a subclass of phenolics, are known to be synthesised by plants in response to microbial infection and they have been found *in vitro* to be effective antimicrobial substances against a

wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Cowan 1999). Tannins and flavonoids are thought to be responsible for antidiarrhoeal activity by increasing colonic water and electrolyte reabsorption (Palombo 2006).

Terpenoids are condensation products of C5 isoprene units which are important constituents of essential oils (Pichersky and Gershenzon 2002). They have been shown to be active against bacteria, fungi, viruses, and protozoa. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds (Cowan 1999).

Alkaloids are the best known nitrogen-containing metabolites of plants and are sparsely distributed in the plant kingdom, but much more specific to defined plant genera and species. This is probably due to the limited supply of nitrogen in plants (Harborne 1999). Alkaloids have been found to have antimicrobial properties with microbicide effects against *Giardia* and *Entamoeba* species as well as antidiarrhoeal effects, which are probably due to their effects on transit time in the small intestine (Cowan 1999).

In research carried out by Nitta et al. (2002), the active extracts obtained from the bark of *Shorea hemsleyana* and roots of *Cyphostemma bainessi* were separated into their components and these exhibited strong inhibitory activity against methicillin-resistant *Staphylococcus aureus*. These active compounds were identified as stilbene derivatives.

2.5 SIGNIFICANCE OF ANTIMICROBIAL SUSCEPTIBILITY TESTING

In screening new antimicrobials or antibiotics, evaluation of biological activity is essential for the assessment of susceptibility of pathogens to the antimicrobial agent. Antimicrobial susceptibility testing is used in pathology to determine the resistance of certain microbial strains to different antimicrobials and in pharmacology research it is used to determine the efficacy of novel antimicrobials from biological extracts against different microorganisms (Das et al. 2010). Microbial growth or its inhibition can be measured in a number of ways, e.g. viable counts, direct microscopic counts, turbidity measurement, bioluminescence and fluorimetry (Grare et al. 2008). Of the various antimicrobial susceptibility methods employed, the disk diffusion method and the broth microdilution method are commonly used to evaluate the effect of the plant extracts or any other antimicrobial on disease-causing pathogens.

The disk diffusion method is used in determining the zones of inhibition exhibited by the plant extracts, while the broth microdilution method, which has been recommended by the Clinical and Laboratory Standards Institute (2003), is used in determining the minimum inhibitory concentration (MIC) of plant extracts. This method is less cumbersome, less expensive and quite reproducible when compared with the disk diffusion method. The use of microplates allows large amounts of data to be generated quickly. Bacterial growth could be assessed either visually by grading turbidity or better spectrophotometrically by measuring optical density (Grare et al. 2008). The disadvantage of visual assessment of bacterial growth is that it lacks objectivity and precision; whereas the accuracy of spectrophotometric readings may be hampered by (i) additives or antibacterial compounds that affect the spectral characteristics of growth media, (ii) the aggregation of bacteria, or (iii) bacterial pigments (Eloff 1998). Colorimetric methods therefore could represent an alternative

approach, using tetrazolium salts as indicators, since bacteria convert them to coloured formazan derivatives that can be quantified (Grare et al. 2008).

2.6 EXTRACTION TECHNIQUES OF PLANT EXTRACTS

In the analysis of medicinal plants, extraction is the crucial first step because it is necessary to extract the desired chemical components from the plant materials for further separation and characterisation (Sasidharan et al. 2011). Different extraction techniques are available, but the most common ones used in plants extraction are the conventional techniques. In conventional extraction, the release of the desired compounds traditionally requires soaking and maceration in mild solvents (Chan et al. 2012). Decoction in water is broadly employed in traditional Chinese medicinal practices and is an effective method that can be considered in cases where the presence of a chemical solvent is undesirable (Das et al. 2010). Other solvents that can be used in conventional extraction are acetone, petroleum ether and hexane. Liquid nitrogen has also been used as a form of extraction in some research work (Karuna et al. 2000). Techniques such as lyophilization (Chen et al. 2003; Grover et al. 2000) and sonification (Chukwujekwu et al. 2009; Yang et al. 2009) are further methods that can be employed other than solvent extraction.

Non-conventional methods that can be used are the supercritical fluid extraction and microwave-assisted techniques. In research carried out by Taiwanese research teams, supercritical fluid extraction was used to investigate the antioxidant activity of the extract of lotus gem (Li et al. 2009). Microwave-assisted extraction has also been used to investigate the bioactivity of tea flower polysaccharides (Wei et al. 2010).The advantages presented by these two non-conventional techniques are short extraction time and solvent-free active compounds.

2.7 ISOLATION AND IDENTIFICATION METHODS

Once the plant extracts have been obtained, identification and characterisation of bioactive compounds becomes a big challenge, because most plant extracts occur as a combination of various types of bioactive compounds or phytochemicals with different polarities. Phytochemical screening assay is a simple, quick, and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemicals or secondary metabolites found in plants (Sasidharan et al. 2011). In isolation of these bioactive compounds, different chromatographic separation techniques, such as thin layer chromatography (TLC), column chromatography, flash chromatography, Sephadex chromatography and high performance liquid chromatography (HPLC), may be used to obtain pure compounds. TLC is a favourite method of most researchers because it gives a quick answer as to how many components are there in a mixture. TLC is also used to support the identity of a compound in a mixture when the retention factor (Rf) of a compound is compared with the Rf of a known compound (Sasidharanet al. 2011). The pure compounds are then used for the determination of structure and biological activity. Numerous analytical methods have been developed, which may facilitate structural determination of the bioactive compound, including TLC, HPLC, LC/electrospray ionisation tandem mass spectrometry (MS/MS), capillary electrophoresis, ion spray mass spectrometry (MS), gas chromatography/MS (GC/MS), and nuclear magnetic resonance (Jeong et al. 2012).

MS provides highly specific chemical information that is directly related to the chemical structure, such as accurate mass, isotope distribution patterns for elemental formula determination and characteristic fragment ions for structural elucidation or identification through spectral matching to authentic compound data.

Moreover, the high sensitivity of MS allows detection and measurement of picomole to femtomole levels of many primary and secondary metabolites (Lei et al. 2011; Sumner et al. 2003).

Non-chromatographic techniques such as immunoassay, which use monoclonal antibodies, phytochemical screening assay or Fourier-transform infrared spectroscopy, can also be used to obtain and facilitate the identification of the bioactive compounds (Sasidharan et al. 2011).

2.8 CURRENT TRENDS IN PHYTOCHEMISTRY AND MEDICINAL PLANT RESEARCH

Synthesis of secondary metabolites by plants is often with highly complex structures. Most of these important secondary metabolites are obtained from wild or cultivated plants because their chemical synthesis is not economically feasible. Various biotechnological methods have been employed in producing some of the secondary metabolites of plants through plant cell cultures. However, this has had limited success because of lack of understanding of how these metabolites are synthesised. State-of-the art genomics tools, however, can be used to enhance the production of known target metabolites or to synthesise entire novel compounds by so-called combinatorial biochemistry in cultivated plant cells (Oksman-Caldenteya and Inzé 2004).

Some plant cells have been used as factories to produce some secondary metabolites. Examples of these are paclitaxel, an anti-cancer drug originally extracted from the bark of 50–60-year-old Pacific yew trees (*Taxus brevifolia*); shikonin, produced by cell suspension cultures of *Lithospermum erythrorhizon;* berberine, produced by cell cultures of *Coptis japonica;* rosmarinic acid, produced by

cell cultures of *Coleus blumeii,* which has been achieved on a large scale, and sanguinarine, produced by cell cultures of *Papaver somniferum,* which has market potential in oral hygiene products (Oksman-Caldenteya and Inzé 2004).

2.9 RELEVANCE OF THIS RESEARCH

The following plants have been reported to be used traditionally in treating diarrhoea; seeds, pulp and leaves of *A. digitata,* bark of *G. livingstonei,* and *S. birrea* (FAO 1993; De Caluweet al. 2009; Masola et al. 2009; De Wet et al. 2010; Gouwakinnou et al. 2011), as seen in Table 1.0. Determination of antibacterial activity and the active components of these plants will provide baseline information on potential usage of extracts from these plants for the treatment of infectious diarrhoea caused by the following bacteria: *Escherichia coli, Staphylococcus aureus, Klebsiella oxytoca, Salmonella enterica* and *Shigella sonnei*. The information obtained from this research could then be used further in developing drugs from these plants or synthesising drugs that mimic the active components in these plants.

Table 1.0 Anti-diarrhoeic Uses of Different Plant Parts

Plant species	Local names	Part used	Preparation	References
Adansonia digitata	Muvhuyu	Seeds	Decoction	FAO (1993)
		Pulp	Decoction	FAO (1993)
		Leaves	Decoction, infusion	(Shahat 2006)
Garcinia livingstonei	Muphiphi, Umphimbi	Bark, root	Crushed, mixed with warm or hot water.	(Kaikabo et al. 2008; De Wet et al. 2010)
Sclerocarya birrea	Mufula, Umganu	Bark	Decoction drunk	(Mathabe et al. 2006; De Wet et al. 2010)

2.9.1 *Adansonia digitata*

A. digitata is one of the eight species of baobab in the genus *Adansonia L.* (Malvaceae, subfamily Bombacoideae) and the only one that occurs naturally on mainland Africa. It is found throughout most of Africa, south of the Sahara (Baum 1995b). In South Africa the population is limited to the Limpopo River Valley, with the exception of a few isolated trees found further south (Wickens and Lowe 2008).

Phylogeographic research shows that baobabs originated in West Africa and spread by human-assisted dispersal to the rest of Africa. Three distinct groups of baobabs have been identified, two in West Africa and one in Southern and Eastern Africa. The baobabs in Southern and Eastern Africa can be regarded as one phylogeographic population owing to low genetic variation within this group (Tsy et al. 2009).

The baobab tree is clearly distinguishable from the other *Adansonia* species endemic in Madagascar and Australia, mainly by its very large trunk (up to 10 m in

diameter), its pendular flowers and its rounded crown. It produces 150 to 300 g dry fruit with a woody epicarp, most of the time ovoid, called “monkey bread”. These fruits contain many seeds in a whitish and floury pulp. The fruit consists of 14 to 28% of pulp with low moisture content; it is acidic, starchy, and rich in vitamin C, calcium and magnesium (Aïda Gabara et al. 2006). The compounded leaves consist of five to seven digitate leaflets. The baobab distribution area is very large. Pollination is done by bats. The tree can be propagated by seeding or vegetative multiplication.

After separating the seeds, the pulp is traditionally used as an ingredient in various preparations or to make beverages. After cooking or grilling, it is either directly consumed or used like thickeners in powder form. The leaves are rich in vitamins (especially C and A) and in iron, and contain mucilage. Very young leaves can be consumed as vegetables, but they are often dried and then reduced to powder (Aïda- Gabara et al. 2006).

Medicinal Uses:

A. digitata is known for its medicinal properties. The flesh of the fruit of the baobab tree is eaten raw as a treatment for diarrhoea and dysentery (Palombo 2006). In Tanzania, the fruit and seeds are prepared as decoction and used in treating dysentery, fever, haemoptysis and diarrhoea (FAO 1993). Leaves of this plant can also be used in treating stomach-ache, diarrhoea, and malaria in cows (De Caluwé et al. 2009), while in South Africa leaves are prepared as infusion and used in treating diarrhoea, fever, inflammation, asthma, kidney and bladder diseases and for blood clearing (Van Wyk and Gericke 2000).

Previous Studies:

Previous studies on the methanolic extract of *A. digitata* roots have reported that it has anti-trypanosomal activity against *Trypanosoma congolense* and *T. brucei* (Atawodi et al. 2003). Stem and root barks of *A. digitata* also contain bioactive constituents such as tannins, phlobatannins, terpenoids, cardiac glycosides and saponins in the stem bark, as well as terpenoids in the aqueous extract of root bark, which are responsible for significant antibacterial activity of the crude extracts of this plant (Masola et al. 2009).

(a) (b)

Fig 1.0 (a) *Adansonia digitata* tree (courtesy: Venter and Witkowski 2010) (b) *Adansonia digitata* fruit

(c) (d)

Fig 1.0 (c) *Adansonia digitata* seeds (d) *Adansonia digitata* fruit pulp

2.9.2 *Garcinia livingstonei*

G. livingstonei T. Anderson (Clusiaceae), commonly known as African Mangosteen or Imbe, is a widespread plant in the warmer parts of Africa, from north of Durban as

far as Somalia and Guinea. In southern Africa, it is distributed widely in the Limpopo and Zambezi Valleys (Johns et al. 1996; National Research Council 2008).

In South Africa, it is found in scrub, open woodland and forest; in Zimbabwe, usually along rivers in the lowveld and frequently in riparian and Munga areas, Mopane woodland and termite mounds in Zambia. It is also found on rocky soil away from water and in open coastal forest (Orwa et al. 2009).

G. livingstonei is a small tree that grows to 18 m and bears small (10−40 mm diameter) yellowish-orange fruits containing a sticky juice. The fruit is edible and has a pleasant flavour. The pulp can be eaten fresh, made into a jam or jellies, or used to prepare ice cream or alcoholic beverages (National Research Council 2008; Gene 2004; Glen 2007).

Medicinal Uses:

G. livingstonei is used for the treatment of diarrhoea by a rural community in northern Maputaland located in KwaZulu-Natal Province, South Africa. Roots and bark are crushed and mixed with warm or hot water, which is administered orally or anally. An infusion (125 ml) is drunk three times a day, until the diarrhoea subsides (De Wet et al. 2010).

Previous Studies:

Acetone extracts of leaves of *G. livingstonei* have been studied for antibacterial activity. Bioautographs showed that two compounds were mainly responsible for the antibacterial activity and these were tested against nosocomial pathogens, namely *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis* and *Pseudomonas*

aeruginosa. Three of the tested organisms were sensitive to both compounds, except *P. aeruginosa,* which was resistant (Kaikabo et al. 2009).

Methanol extracts of root bark of *G. livingstonei* have also shown antiparasitic activity against some selected parasites (Mbwambo et al. 2006).

(a) (b)

Fig 2.0 (a) *Garcinialivingstonei* tree (b) *Garcinialivingstonei tree* bearing fruits (courtesy: Glen 2007)

2.9.3 *Sclerocarya birrea*

S. birrea (Anacardiaceae) is a fast-growing tree. Three subspecies of *S. birrea* are known. The subspecies *caffra* occurs mainly in the southern part of Africa and is known as marula. The subspecies multifoliolata is restricted to Tanzania and possibly the neighbouring part of Kenya and the subspecies *birrea* is present in Western and Central Africa (Nghitoolwa et al. 2003). Flowering takes place in the dry

season when trees are leafless. The major pollinators (or flower visitors) of *S. birrea* are honey bees. Secondary pollinators include flies and wasps (Chirwa and Akinnifesi 2008). *S. birrea* bears plum-sized stone fruit with a thick yellow peel and translucent white flesh. Many are eaten fresh, but most are processed into products such as beverages, jams and jellies. Regardless of taste (sweet-and-sour or tart), the juice is reported to be nutritionally important, containing as much as four times the vitamin C of orange juice (National Research Council 2008).

Medicinal Uses:

In traditional medicine, the bark of *S. birrea* is the part most frequently used to treat ailments that are mostly bacteria-related (stomach-aches, diarrhoea, wounds and coughs) (Gouwakinnou et al. 2011). The bark of *S. birrea* subsp. *caffra* is crushed and mixed with hot, warm or cold water. The mixture is administered anally or orally. The mixture (125 ml) is drunk three times a day, until diarrhoea subsides. If administered anally, the dosage depends on the person's weight (Mathabe et al. 2006; De Wet et al. 2008).

Previous Studies:

Previous studies have shown that extracts from the stem bark and leaves of *S. birrea* possess a catalogue of pharmacological activities, including analgesic, anti-inflammatory, anti-diabetic and hypoglycaemic (Ojewole 2004), antidiarrhoeal (Galvez 1991;Galvez 1993), antibacterial (Eloff 2001) and insecticidal properties (Fatope et al. 1993).

Galvez's (1991) investigation of antidiarrhoeic activity of the bark of *Sclerocarya birrea* in rats revealed that the antidiarrhoeic activity was related to an inhibition of

intestinal transit rather than to inhibition of net secretion of fluid and electrolytes provoked by the laxative agents used. A condensed tannin was isolated from the crude drug which produced inhibition in intestinal motility, and the monomer was identified as procyanidin.

Eloff (2001) reported antibacterial activities against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* using acetone extracts of bark and leaves of *Sclerocarya birrea* with MIC values from 0.15 to 3 mg/ml.

(a) (b)

Fig 3.0 (a) *Sclerocarya birrea* tree (b) *Sclerocarya birrea* treebearing fruits (courtesy: Mutshinyalo and Tshisevhe 2003)

(c)

Fig 3.0 (c) *Sclerocarya birrea* bark (courtesy: Mutshinyalo and Tshisevhe 2003)

2.10 HYPOTHESIS AND OBJECTIVES

HYPOTHESIS

1. Crude extracts of *A. digitata* seeds, pulp and leaves, *G. livingstonei* and *S. birrea* barks will show antibacterial activities against bacteria causing diarrhoea, such as *Escherichia coli, Staphylococcus aureus, Klebsiella oxytoca, Salmonella enterica* and *Shigella sonnei.*

2. The crude extract of the plants that show antimicrobial activity will have active components with antibacterial properties.

OBJECTIVES

1. To determine the susceptibility of *Escherichia coli, Staphylococcus aureus, Klebsiella oxytoca, Salmonella enterica,* and *Shigella sonnei* to the crude extracts of *A. digitata*, *G. livingstonei* and *S. birrea*.

2. To determine the MIC and minimum bactericidal concentration (MBC) of *A. digitata*, *G. livingstonei*, and *S. birrea* plant extracts on the test bacteria.

3. To carry out cytotoxicity tests on plant extracts with most antimicrobial properties.

4. To determine the antioxidant properties and phytochemical analysis of the plant extracts.

CHAPTER 3: ANTIMICROBIAL AND CYTOTOXICITY ASSAYS

3.1 MATERIALS AND METHOD

3.1.1 PLANT MATERIALS AND SAMPLE COLLECTION

The leaves, fruit pulps and seeds of *A. digitata,* bark of *G. livingstonei* and *S. birrea* were collected from Venda, Limpopo Province of South Africa, and voucher specimens were prepared and identified at the H.G.W.J Schweikerdt Herbarium, University of Pretoria.

3.1.2 INSTRUMENTS AND REAGENTS USED

Grinding machine, shaker, rotavapor, freeze drier, beakers, conical flasks, round-bottom flasks, vacuum filter, filter papers, methanol and water.

3.1.3 PREPARATION OF PLANT EXTRACT

The method described by Ndip et al. (2007) was employed; different plant parts were harvested, air-dried for about two weeks and ground to a fine powder. Methanol (100%) and water were used as solvents for extraction of crude extract. Water was chosen as a solvent so as to mimic the traditional style, since most of these plant parts were administered as either infusions or decoctions. Twenty grams of each powdered plant material was macerated in 200 ml of each solvent in extraction pots such that the level of the solvent was above that of the plant material. The macerated mixtures were then left on the shaker for 72 hours at room temperature. The extracts were filtered out from the macerated mixture by vacuum filtration. The methanol extracts were concentrated in a Buchi Rotavapor R-200, transferred to appropriately labelled vials and allowed to stand at room temperature to permit evaporation of residual solvents. The water extracts were concentrated using a freeze dryer.

(a)

(b)

Figure 4.0 (a) Rotavapor used to concentrate the methanol extracts (b) Freeze dryer used to concentrate the water extracts

3.2 ANTIMICROBIAL ACTIVITY

3.2.1 INSTRUMENTS AND REAGENTS/MATERIALS USED

Autoclave, laminar flow with Bunsen burner, spectrophotometer, weighing machine, inoculating loop, Pasteur pipettes, Petri dishes, pipettes, 96-well microtitre plates, 50 ml and 100 ml bottles, 1 ml and 2 ml Eppies, barium chloride, sulphuric acid, dimethyl sulphoxide (DMSO), Mueller-hinton agar, nutrient broth, presto blue, ciprofloxacin, water and methanol plant extracts.

3.2.2 PREPARATION OF BACTERIA CULTURE

The microorganisms used were *Staphylococcus aureus* (ATCC 25923)*, Escherichia coli* (ATCC 8739)*, Klebsiella oxytoca* (ATCC 49131)*, Salmonella enterica* subsp. *Typhimurium* (ATCC 14028) and *Shigella sonnei* (ATCC 25931). Most of these bacteria have been extensively implicated in infectious diarrhoea. Fresh bacterial

cultures were prepared by sub-culturing stock bacterial cultures into freshly prepared nutrient agar and incubating at 37^0C for 24 hours. These 24-hour-old bacterial cultures were transferred into freshly prepared nutrient broth and standardised to 0.5 McFarland turbidity standards using the spectrophotometer to obtain the desired cell density of 1.5 X 10^8 (cells/ml).

The 0.5 McFarland turbidity standard was prepared by adding 0.05 ml of 1.175% of barium chloride dihydrate ($BaCl_2$•$2H_2O$), with 9.95 mL of 1% sulphuric acid (H_2SO_4).

3.2.3 AGAR WELL DIFFUSION ASSAY

The Agar Well Diffusion Assay was employed with modifications as described by Irshad et al. (2012). The Agar Well Diffusion medium was prepared by pouring molten Mueller-Hinton agar on petri dishes and allowing it to solidify. Afterwards, 100µl of inoculums, approximately 1.5 X 10^8 cells /ml was seeded into warm molten Mueller-Hinton agar and poured on the surface of the solidified agar. This was allowed to solidify and holes of 5 mm width were made into the agar using sterile Pasteur pipettes.

An amount of 100 mg/ml stock of crude plant extract was prepared for each plant by dissolving 100 mg of dried plant extract in 1 ml of 10% DMSO. 100 µL of the stock extract was pipetted onto the holes to give a concentration of 10 mg per hole. 100 µL of 0.5 mg/ml ciprofloxacin was also pipetted into one of the holes to give a final concentration of 0.05 mg. This served as the positive control, while 100 µL of 10% DMSO was pipetted into one of the holes, which served as the negative control.

3.2.4 BROTH MICRODILUTION ASSAY FOR MIC AND MBC

The MIC of the plant extracts was determined with some modification, as described by Eloff (1998). 100 µL of nutrient broth was added to all the wells of a 96-well

microtitre plate. 100 µL of each dissolved plant extract (50mg/ml) was then added in triplicate for each bacterial plate on the first rows (row A). These were serially diluted row by row and 100 µL of the mixture was discarded from the last row, thus leaving each diluted well with a volume of 100 µL. The same procedure was carried out for ciprofloxacin (positive control), during which 100 µL of 2.5 mg/ml of dissolved ciprofloxacin was added in triplicate to row A. 100 µL of each bacterial suspension in suitable growth medium (nutrient broth) was then added to all the wells except the last column, which served as the sterile control (containing 200 µL of nutrient broth).

Wells containing bacterial suspensions and growth medium, as well as wells containing 10% DMSO, bacteria suspensions and growth medium, were used as negative control. The microtitre plates were incubated at 37°C for 24 hours and the MICs were the lowest concentration where no viability was observed after 24 hours on the basis of metabolic activity. To indicate respiratory activity, a change in colour from blue to pink would be determined after adding 20 µL of Presto blue per well in duplicate and incubating it at 37°C for 30 min.

In order to determine the MBC, the method described by Eloff (1998) was also employed: 150 µL of nutrient broth was pipetted into all the wells of a 96-well microtitre plate, and 50 µL was taken from the 24-hour-old suspension of the undisturbed column (the column without Presto blue) in the MIC plates and added to the freshly prepared microtitre plates to make up a final volume of 200 µL. These were incubated at 37^0C for 24 hours.

After 24 hours, the plates were taken out of the incubator and 20 µL of Presto blue was added to observe for colour change. The MBC would be the concentration where no change in colour was observed.

3.3 CYTOTOXICITY EXPERIMENT

3.3.1 INSTRUMENTS AND REAGENTS/MATERIALS USED

Weighing machine, microscope, laminar flow, Pasteur pipettes, 96-well microtitre plates, 24-well microtitre plates, 50 ml and 100 ml bottles, 1 ml and 2 ml Eppies, pipettes, ELISA plate reader, Dulbecco's Modified Eagle's Medium (DMEM), Foetal bovine serum (FBS), penicillin/streptomycin (PS), DMSO, XTT reagent, actinomycin D, human embryonic kidney (HEK) 293 cells, water and methanol plant extracts.

3.3.2 XTT CYTOTOXICITY ASSAY

The cytotoxicity of water and methanol extracts of *G. livingstonei* and *S. birrea* were tested on Human Embryonic Kidney (HEK) 293 cells. 100 µL of HEK cell suspension (1×10^5 cells/ml) was added to the inner wells of a 96-well plate and 200 µL of incomplete medium (Dulbecco's modified eagle's medium(DMEM)) to the outer wells. The plates were incubated for 24 hours to allow the cells to attach to the base of the plate. Serial dilution of the plant extracts (already dissolved in DMSO), positive control (Actinomycin D) and negative control (DMSO) with the complete medium (10% foetal bovine serum + DMEM + Penicillin/Streptomyosin) were carried out in a 24-well plate to give eight different concentrations of each sample and a final volume of 1ml per well.

100µL of each concentration from the 24-well plate was added to the 96-well plate in triplicate and there was a triplicate medium control and a DMSO control for each extract. A reference plate was prepared to account for the colour of the plant extracts. This plate contained the plant extracts and medium in duplicate but no cells. The plates were incubated for 72 hours, after which 50 µL of XTT reagent from ROCHE was added to all the wells and they were incubated for about 2 h 30 min.

After incubation the plates were read on an ELISA plate with KC Junior software to read the absorbance at 450 nm and 690 nm as the reference wavelength. Graph pad prism software was used to analyse the data.

3.4 RESULTS

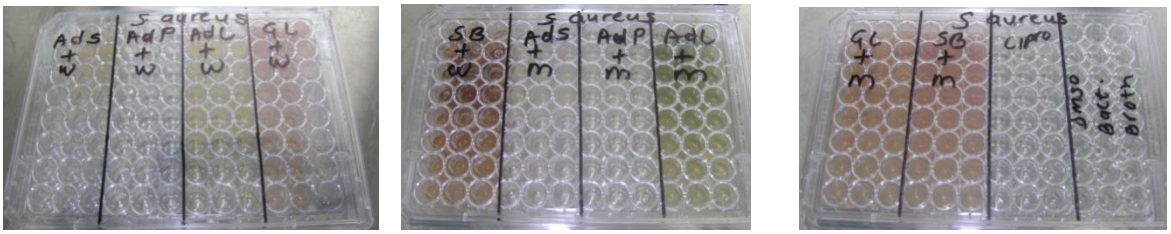
ZONES OF INHIBITION

The water extracts of *A. digitata* seeds, pulp and leaves, as well as methanol extracts of *A. digitata* seeds and pulp, showed no zones of inhibition on all the test bacteria in the agar well diffusion assay. The methanol extract of the leaves of *A. digitata* however, inhibited the growth of *S. aureus*. Both the water and methanol extract of *G. livingstonei* showed zones of inhibition for *S. aureus* and *S. sonnei.* In addition to these two, the methanol extract also showed zones of inhibition for *K. oxytoca.* The *G. livingstonei* extracts did not show any inhibition against *E. coli* and *S. enterica*. Water and methanol extract of *S. birrea* showed zones of inhibition for all the test organisms.

MIC

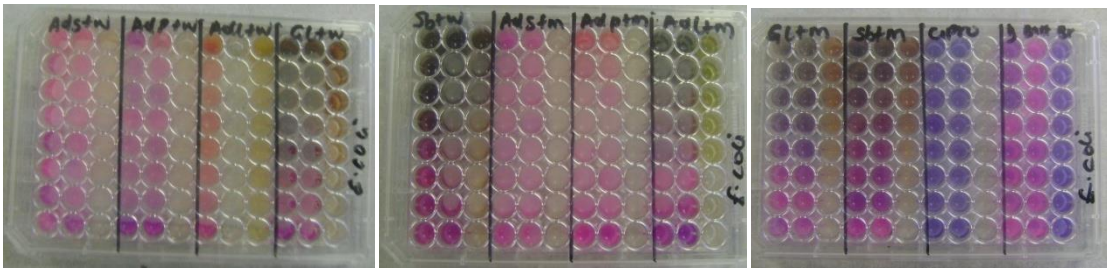
The broth microdilution assay showed that the water and methanol extracts of *A. digitata* seeds and pulp had MICs greater than 12.5mg/ml. The water extract of *A. digitata* leaf had MIC of 1.56mg/ml for all the test bacteria except for *S. sonnei,* with an MIC of 6.25 mg/ml (Table 2.0). Methanol extract of *A. digitata* leaf had MIC of 1.56 mg/ml for all the test bacteria, except for *S. aureus* with an MIC of 0.78 mg/ml and *K. oxytoca* with an MIC of 0.39 mg/ml. Water extract of *G. livingstonei* bark had an MIC of 1.56 mg/ml for all the test organisms except for *E. coli,* and *S. sonnei* which had MIC's of 0.78 mg/ml, while the methanol extract of *G. livingstonei* bark

had an MIC of 0.39 mg/ml for all the test organisms with the exception of *E. coli,* and *S. sonnei* which maintained MIC's of 0.78 mg/ml. Water extract of *S. birrea* bark had an MIC of 0.78 mg/ml for all the test bacteria except *E. coli* and *S. enterica* which had lower MIC's of 0.39 mg/ml, while the methanol extract of *S. birrea* bark had varied MIC values ranging from 0.39 to 1.56 mg/ml (Table 2.0).



(a) (b) (c)

Figure 5.0 Images of micro titre plates with plant extracts and test organism, as well as the positive and negative controls before incubation



(a) (b) (c)

Figure 6.0 Images of micro titre plates with plant extracts and test organism, as well as the positive and negative controls after 24 hours incubation and addition of Presto blue.

Table 2.0: Minimum Inhibitory Concentration Results of Plant Extracts (mg/ml)

	Plant Extract						
Bacteria	**Adlw**	**Adlm**	**Gabw**	**Gabm**	**Scbw**	**Scbm**	**Cipro**
S. aureus	1.56	0.78	1.56	0.39	0.78	0.39	0.002
E. Coli	1.56	1.56	0.78	0.78	0.39	1.56	0.002
K. oxytoca	1.56	0.39	1.56	0.39	0.78	0.78	0.002
S. enterica	1.56	1.56	1.56	0.39	0.39	0.39	0.002
S. sonnei	6.25	1.56	0.78	0.78	0.78	0.78	0.002

Key: **Adsw**= water extract of *Adansonia digitata* seeds, **Adsm**= methanol extract of *Adansonia digitata* seeds, **Adpw**= water extract of *Adansonia digitata* pulp, **Adpm**=methanol extract of *Adansonia digitata* pulp, **Adlw**= water extract of *Adansonia digitata* leaves, **Adlm**= methanol extract of *Adansonia digitata* leaves, **Gabw**= water extract of *Garcinia livingstonei* bark, **Gabm**= methanol extract of *Garcinia livingstonei* bark, **Scbw**= water extract of *Sclerocarya birrea* bark, **Scbm**= methanol extract of *Sclerocarya birrea* bark, **Cipro** = Ciprofloxacin. NB: Adsw, Adsm, Adpw and Adpm had MIC values >12.5 mg/ml, figures not presented in the table.

MBC

The water and methanol extracts of *A. digitata* seeds, pulp and leaves had MBCs greater than 12.5 mg/ml, while the water extract of *G. livingstonei* bark had an MBC of 12.5 mg/ml for all the test bacteria (Table 3.0). Similarly, the methanol extract of *G. livingstonei* bark had an MBC of 12.5 mg/ml for the other test bacteria except for *S. aureus,* with an MBC of 6.25 mg/ml and *S. enterica* with an MBC greater than 12.5 mg/ml (Table 3.0).

The methanol extract of *S. birrea* bark had an MBC of 12.5 mg/ml for all the test bacteria except *S. sonnei,* with an MBC of 6.25 mg/ml. On the other hand, water extract of *S. birrea* bark also had an MBC of 6.25 mg/ml for all the test organisms (Table 3.0).

Table 3.0: Minimum Bactericidal Concentration Results of Plant Extracts (mg/ml)

Bacteria	**Plant Extracts**				
	Gabw	**Gabm**	**Scbw**	**Scbm**	**Cipro**
S. aureus	12.5	6.25	6.25	12.5	0.002
E. Coli	12.5	12.5	6.25	12.5	0.002
K. oxytoca	12.5	12.5	6.25	12.5	0.002
S. enterica	12.5	>12.5	6.25	12.5	0.002
S. sonnei	12.5	12.5	6.25	6.25	0.002

Key: **Adsw**= water extract of *Adansonia digitata* seeds, **Adsm**= methanol extract of *Adansonia digitata* seeds, **Adpw**= water extract of *Adansonia digitata* pulp, **Adpm**=methanol extract of *Adansonia digitata* pulp, **Adlw**= water extract of *Adansonia digitata* leaves, **Adlm**= methanol extract of *Adansonia digitata* leaves, **Gabw**= water extract of *Garcinia livingstonei* bark, **Gabm**= methanol extract of *Garcinia livingstonei* bark, **Scbw**= water extract of *Sclerocarya birrea* bark, **Scbm**= methanol extract of *Sclerocarya birrea* bark, **Cipro** = Ciprofloxacin. NB: Adsw, Adsm, Adpw, Adpm, Adlw, and Adlm had MBC values >12.5 mg/ml, figures not presented in the table.

CYTOTOXICITY

The cytotoxicity results are expressed as EC_{50} which is the maximal effective concentration needed to kill fifty percent of the HEK cells. The water extract of *G. livingstonei* bark had the highest EC_{50} value of 769.9µg/ml ± 36.33, followed by the methanol extract while the methanol extract of *S. birrea* bark had the lowest EC_{50} value of 105.9µg/ml ±19.50, followed by its water extract (Table 4.0). Figures 7.0-10.0 show a trend of decreasing cell proliferation with an increase in the plant extracts concentration.

Table 4.0: The Half Maximal Effective Concentration of Four Plant Extracts and Actinomycin D on HEK Cell Lines

Plant Extract	**EC_{50} (µg/ml)**	**R^2 value**
Gabw	769.9µg/ml ± 36.33	0.35
Gabm	> 400µg/ml	0.89
Scbw	149.3µg/ml ±1.4	0.99
Scbm	105.9µg/ml ±19.50	0.96
Act	0.003±0.000095	0.88

Key: **Gabw**= water extract of *Garcinia livingstonei* bark, **Gabm**= methanol extract of *Garcinia livingstonei* bark, **Scbw**= water extract of *Sclerocarya birrea* bark, **Scbm=** methanol extract of *Sclerocarya birrea* bark, **Act** = Actinomycin D.

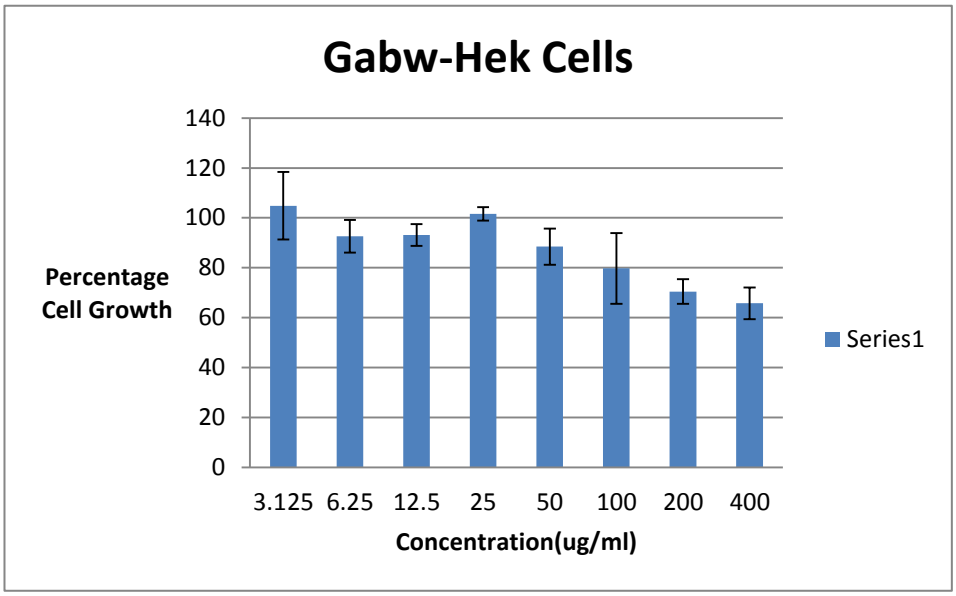


Figure 7.0 Chart showing the inhibitory activity of water extract of *G. livingstonei* on HEK cells. X axis =Concentrations of water extract of *G. livingstonei* bark Y axis = Percentage growth of HEK cells.

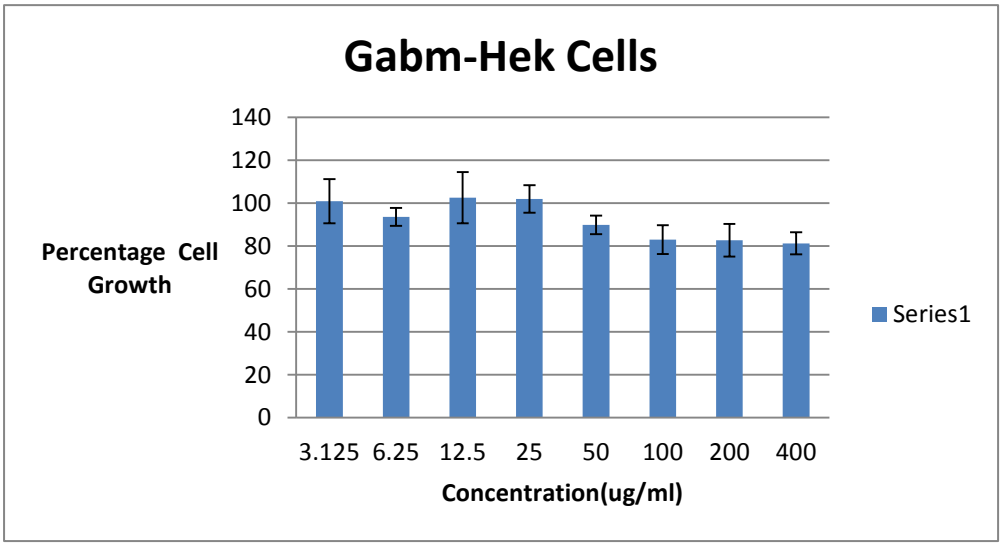


Figure 8.0 Chart showing the inhibitory activity of methanol extract of *G. livingstonei* bark on HEK cells. X axis =Concentrations of methanol extract of *G. livingstonei* bark; Y axis = Percentage growth of HEK cells.

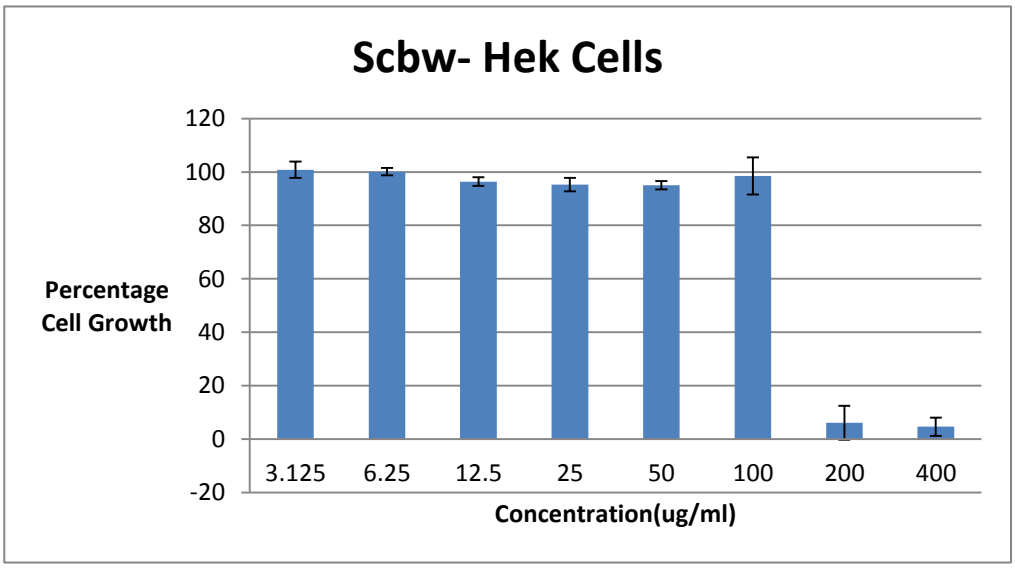


Figure 9.0 Chart showing the inhibitory activity of water extract of *S. birrea* bark on HEK cells. X axis =Concentrations of water extract of *S. birrea* bark; Y axis = Percentage growth of HEK cells.

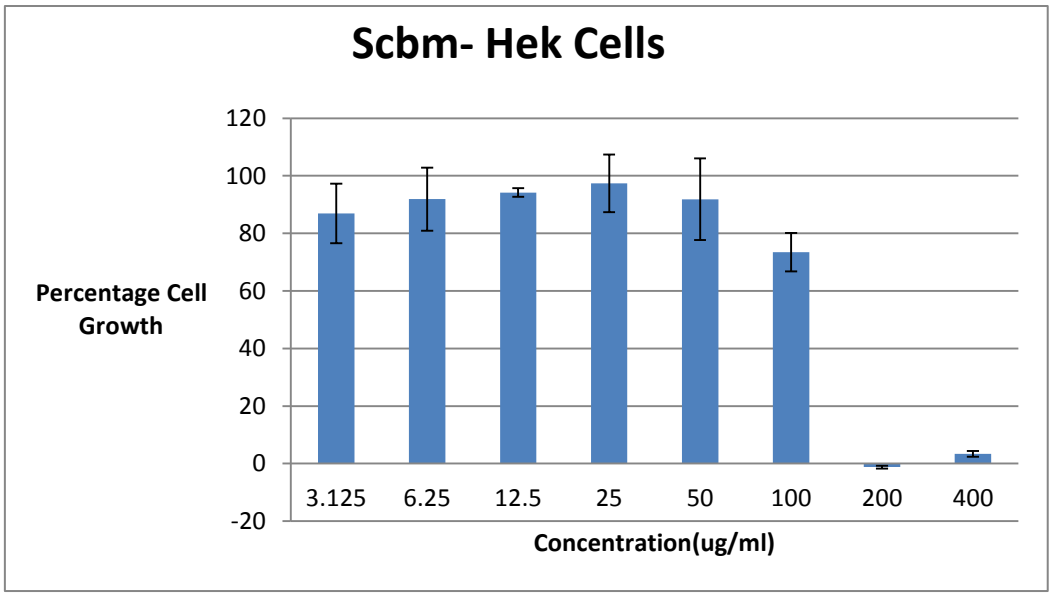


Figure 10.0 Chart showing the inhibitory activity of methanol extract of *S. birrea* bark on HEK cells. X axis =Concentrations of methanol extract of *S. birrea* bark; Y axis = Percentage growth of HEK cells.

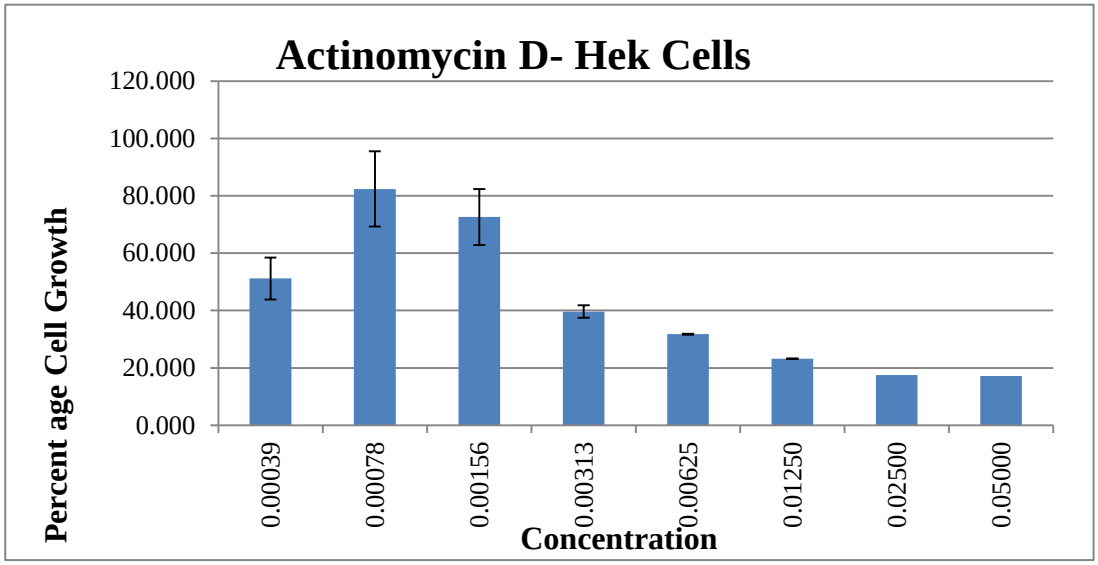


Figure 11.0 Chart showing the inhibitory activity of the positive control Actinomycin D on HEK cells. X axis =Concentrations of Actinomycin D; Y axis = Percentage growth of HEK cells.

Therapeutic Index

The therapeutic index (TI) is determined as the ratio of the cytotoxicity to that of the minimum inhibitory concentration. It is a measure of the safety and efficacy of an antimicrobial agent. The wider the values of the therapeutic index (i.e. large difference between its toxicity and the MIC), the greater the antimicrobial specificity. (Jiang et al. 2014).

Table 5.0: Cytotoxicity and Therapeutic Index of Three Medicinal Plant Extracts against Five Bacterial Pathogens Calculated by Dividing Cytotoxicity by MIC

		Therapeutic index				
Plant Extracts	**Cytotoxicity (mg/ml)**	***S. aureus***	***E. coli***	***K. oxytoca***	***S. enterica***	***S. sonnei***
Gabw	0.7699	0.49	0.98	0.49	0.49	0.98
Scbw	0.1493	0.19	0.38	0.19	0.38	0.19
Scbm	0.1059	0.27	0.067	0.14	0.27	0.14

Key: **Gabw**= water extract of *Garcinia livingstonei* bark, **Gabm**= methanol extract of *Garcinia livingstonei* bark, **Scbw**= water extract of *Sclerocarya birrea* bark, **Scbm**= methanol extract of *Sclerocarya birrea* bark. NB: Therapeutic index for Gabm could not be determined because its cytotoxic value was greater than 400 µg/ml.

3.5 DISCUSSION

In the agar diffusion assay, paper discs (already containing measured plant extracts) were initially used as antimicrobials to carry out the experiment. The results, however, showed little or no zones of inhibition for all the plant extracts, though the experiments were repeated three times in duplicate. The agar well method was then considered and used by making holes in the solidified agar instead of paper discs and zones of inhibition were observed for some of the plant extracts.

The water and methanol extracts of *A. digitata* seeds and pulp showed no antimicrobial activity against any of the test bacteria. This could be due to the fact that water and methanol as solvent could not extract the antibacterial compounds

present in these plant parts, considering that inhibition of *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus mutans* and *Pseudomonas aeruginosa* by the ethyl acetate and n-butanol extracts of the pericarp, pulp and seed portion of *A. digitata* has been reported (Shukla et al. 2003).

Contrary to the agar well diffusion assay in which methanol and water extract of *A. digitata* leaves showed little and no inhibition respectively, the broth microdilution method indicated that both the water and methanol extracts of *A. digitata* leaves exhibited inhibitory activities against all of the test bacteria at concentrations of 0.39 mg/ml to 6.25 mg/ml. Their bactericidal effect, however, was at concentrations greater than 12.5 mg/ml. The methanol extracts of *A. digitata* root bark and leaves have been shown to exhibit antibacterial activity against *Staphylococcus aureus, Streptococcus faecalis, Bacillus subtilis, Escherichia coli* and *Mycobacterium phlei* (Anani et al. 2000).

The disadvantage of using the agar diffusion method to determine antimicrobial activity is that the antimicrobial effect may be affected by the agar type, salt concentration, incubation temperature and molecular size of the antimicrobial component. Furthermore, it does not distinguish between bactericidal and bacteriostatic effects (Eloff 1998).

The MIC results (0.39-1.56mg/ml) showed that the *G. livingstonei* plant extracts displayed antimicrobial activity against all the test bacteria. Elsewhere, two compounds isolated from acetone extracts of *G. livingstonei* leaves were shown to exhibit antimicrobial activity against *Escherichia coli, Staphylococcus aureus* and *Enterococcus faecalis* with MIC's ranging from 8-100 µg/ml, while the methanol

extract of its root bark showed antiparasitic activity against some selected parasites (Kaikabo et al. 2009; Kaikabo and Eloff 2011; Mbwambo et al. 2006).

Water and methanol extracts of *S. birrea* bark showed both significant inhibitory and bactericidal effect on all the test organisms with MIC which ranged from 0.39 to 1.56 mg/ml. This is in line with the findings of Eloff (2001), who reported that acetone extracts of *S. birrea* bark and leaves showed significant antimicrobial activities with MIC values ranging from 0.15 to 3 mg/ml against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis*.

The EC_{50} of all the plant extracts, which ranged from 105.9µg/ml to 769.9µg/ml, was much higher than that of Actinomycin D, the positive control. At this level, these extracts are considered safe, considering that the values are greater than 100 µg/ml, which has been considered safe for extracts of *Rubia cordifolia* roots (Patel et al.2010).

Of all the extracts studied, water extract of *G. livingstonei* bark had the highest therapeutic index of 0.98 for *E. coli* and *S. sonnei*, which is similar to the findings of Adamu et al. (2014) who reported that *Strychnos mitis* extracts had a therapeutic index of 1.1 for *E. coli.*

CHAPTER 4: PHYTOCHEMICAL AND ANTIOXIDANT ASSAYS

4.1 MATERIALS AND METHODS

4.1.1 PREPARATION OF PLANT EXTRACT

The method described in 3.1.3 above was employed in preparing the plant extracts.

4.1.2 INSTRUMENTS AND REAGENTS/MATERIALS USED

Weighing machine, test tubes, spray bottle, 1 ml and 2 ml Eppies, pipettes, filter papers, 10% DMSO, methanol, Wagner's reagent, Dragendroff's reagent, sodium hydroxide, hydrochloric acid, ethyl acetate, aluminium chloride, phoshomolybdic acid reagent, ammonia vapours, alcoholic ferric chloride, chloroform, sulphuric acid, distilled water, water and methanol plant extracts**,** ELISA plate reader, 96-well microtitre plates, foil paper, Vitamin C, ethanol, 1,1-diphenyl-2-picrylhydrazyl (DPPH).

4.2 PHYTOCHEMICAL SCREENING

The methods described below were used to assess the phytochemical constituents of the plant extracts.

4.2.1 TEST FOR ALKALOIDS

WAGNER'S TEST: A fraction of extract was treated with Wagner's test reagent [1.27 g of iodine and 2 g of potassium iodide in 100 ml of water] and observed for the formation of a reddish brown colour precipitate (Lalitha and Jayanthi 2012).

DRAGENDROFF'S TEST: A few drops of Dragendroff's reagent were added to a test tube containing 1 ml of extract and a colour change was observed. The

appearance of an orange colour was an indication of the presence of alkaloids (Firdouse and Alam 2011).

4.2.2 TEST FOR FLAVONOIDS

SODIUM HYDROXIDE TEST: Plant extract is treated with dilute NaOH, followed by addition of dilute HCl. A yellow solution with NaOH turns colorless with dilute HCl, which shows the presence of flavonoids (Onwukaeme et al. 2007). A conclusive test was carried out using the aluminum chloride Test.

ALUMINUM CHLORIDE TEST: A quantity (0.2 g) of each of the extracts was heated with 10 ml of ethyl acetate in boiling water for three minutes, after which 4 ml of the filtrate was shaken with 1 ml of 1% aluminum chloride solution and observed for light yellow colouration. A yellow precipitate indicated the presence of flavonoids (Subash et al. 2013).

4.2.3 TEST FOR PHENOLS

In this test, one spots the extract on a filter paper, adds a drop of phoshomolybdic acid reagent and exposes it to ammonia vapours. Blue colouration of the spot indicates the presence of phenols (Kumar et al. 2007).

4.2.4 TEST FOR TANNINS

In this test 10% alcoholic ferric chloride is added to 2–3 ml of methanolic extract (1:1). Dark blue or greenish grey colouration of the solution reveals the presence of tannins (Kumar et al. 2007; Parekh and Chanda 2007).

4.2.5 TEST FOR TERPENOIDS

To conduct this test, 5 ml of plant extract is added to 2 ml of chloroform and 3 ml of concentrated sulphuric acid. The presence of terpenoids gives a reddish brown colour of interface (Edeoga et al. 2005).

4.2.6 TEST FOR SAPONINS

One adds 0.5 ml of plant extract filtrate to 5 ml of distilled water and shakes it well. Persistence of frothing is an indication of the presence of saponins (Parekh and Chanda 2007).

4.3 DETERMINATION OF ANTIOXIDANT ACTIVITY

The method described by Du Toit et al. (2001) was employed to determine the antioxidant content of the plant extracts as well as that of vitamin C so as to have a basis for comparison. 2mg of plant extract was dissolved in 200µl of ethanol to give a stock concentration of 10mg/ml while 2mg of vitamin C was dissolved in 1ml of ethanol to give a stock concentration of 2 mg/ml which served as the positive control.

1,1-diphenyl-2-picrylhydrazyl (DPPH) which is a free radical that can be reduced by an antioxidant, was prepared by dissolving 20 mg in 500 ml ethanol to give a stock concentration of 0.04 mg/ml. All the samples were tested in triplicate on 96-well microtitre plates. The start-up concentration for the test samples in the first row was 500 µg/ml, which is a composition of distilled water and the extract, while the positive control (vitamin C) was a mixture of distilled water and vitamin C with a final concentration of 100 µg/ml in the first row. Blank controls were prepared by adding ethanol to distilled water and negative controls also had test samples and distilled water. These were serially diluted from the first row to the last row, and DPPH was

added into all the wells except the negative controls. The plates were covered in foil and incubated for 30 minutes. The absorbency were read with an ELISA plate reader (KC junior) at 515 nm and the absorbency values were analysed with the graph pad prism software.

4.4 RESULTS

PHYTOCHEMICALS

The water and methanol extracts of each plant part had the same test result, except for extracts of *S. birrea,* as seen in Table 6.0. Alkaloids were absent in both the water and methanol extracts of *A. digitata* seeds, pulps and leaves. Flavanoids and phenols were present in both the water and methanol extracts of *A. digitata* seeds, pulp and leaves. Tannins were only detected in the leaf extract of *A. digitata,* but were absent in the seed and pulp extracts. Terpenoids were only detected in the pulp extract of *A. digitata* but not in its seed or leaf extracts. Saponins were present in both the pulp and leaf extracts of *A. digitata,* but were not detected in its seed extracts. Alkaloids, flavonoids, phenols, tannins, terpenoids and saponins were present in the bark extracts of *G. livingstonei* and *S. birrea,* with the exception of the water extract of *S. birrea,* which did not contain any flavonoids.

Table 6.0: Phytochemical Analysis of Ten Plant Extracts

	[β]**Ads**	[β]**Adp**	[β]**Adl**	[β]**Gab**	[βκ] **Scb**
[α]**Alkaloids**	-	-	-	+	+
Flavonoids	+	+	+	+	+
Phenols	+	+	+	+	+
Tannins	-	-	+	+	+
Terpenoids	-	+	-	+	+
Saponins	-	+	+	+	+

Ads= *Adansonia digitata* seed extracts**, Adp=** *Adansonia digitata* pulp extracts **Adl=** *Adansonia digitata* leave extracts**, Gab=** *Garcinia livingstonei* bark extracts**, Scb =** *Sclerocarya birrea* bark extract, **+**= positive test, **-** =negative test. **α** = same alkaloid test results for Wagner's and Dragendorf's test, **β** = similar test results for both water and methanol extracts of plants parts, **K**= only the methanol extract was positive for flavonoids

ANTIOXIDANT

G. livingstonei and *S. birrea* bark extracts had the lowest IC_{50} values (that is concentration that inhibits 50% of DPPH), followed by the water and methanol extracts of *A. digitata* leaves (Table 7.0). The water extract of *A. digitata* pulp had the highest IC_{50} value, followed by the water extract of *A. digitata* seed. There was a little difference in the IC_{50} values between the water and methanol extract of *G. livingstonei* bark, but the IC_{50} value of the water extract of *S. birrea* was slightly higher than that of the methanol extract. The IC_{50} values of the water and methanol extracts of the leaves were similar, while the IC_{50} value of the water extracts of *A. digitata* seed and pulp was higher than that of their methanol extracts. The positive control, vitamin C, had an IC_{50} value close to the methanol extract of *A. digitata* pulp.

Table 7.0: 50% Inhibitory Concentration of Ten Plant Extracts and Vitamin C and their Coefficient of Determination Values (R^2)

Plant extracts	**Solvent used**	**IC_{50} (µg/ml)**	**R^2 value**
Ads	Water	23.11± 1.37	0.89
	Methanol	6.65± 1.51	0.79
Adp	Water	28.58± 2.07	0.87
	Methanol	9.65± 2.19	0.74
Adl	Water	2.79±0.07	0.94
	Methanol	2.82±0.05	0.95
Gab	Water	0.35±0.06	0.84
	Methanol	0.39±0.04	0.88
Scb	Water	0.40±0.02	0.93
	Methanol	0.28±0.02	0.88
Vit C	Water	10.62±0.87	0.83

Ads = *Adansonia digitata* seed extracts, **Adp** = *Adansonia digitata* pulp extracts **Adl** = *Adansonia digitata* leave extracts, **Gab** = *Garcinia livingstonei* bark extracts, **Scb** = *Sclerocarya birrea* bark extracts. Values are expressed as mean±SD (n=3). The IC_{50} of vitamin C, the positive control was 10.62±0.87

4.5 DISCUSSION

In the phytochemical assay, the test for flavonoids, using the sodium hydroxide test, did not give a clear indication of the presence or absence of flavonoids. This is due to the colour of some of the dissolved plant extracts, which was similar to the positive colour change for flavonoids. Another similar test had to be carried out, namely the aluminium chloride test, and this gave a clearer indication of the presence or absence of flavonoids.

The fact that water and methanol extracts of all samples yielded the same test results, with the exception of the flavonoid test result of *S. birrea,* is because water and methanol are polar solvents and therefore extract similar compounds. Methanol, on the other hand, could have extracted more polyphenols such as flavonoids better than water (Medini et al. 2014), which explains why the methanol extract of *S. birrea* tested positive for flavonoid. Looking at this test result, the water extract of *S. birrea* is probably not a good source of flavonoids.

Alkaloids were absent in both the water and methanol extracts of *A. digitata* seeds, pulp and leaves. Chemicals that have been isolated and characterised from the *A. digitata* plant usually belong to the classes of terpenoids, flavonoids, steroids, vitamins, amino acids, carbohydrates and lipids (Shukla et al. 2001). This explains why *A. digitata* seeds, pulp and leaves contain flavonoids and phenols, as most phenolic compounds belong to the flavonoid group.

Tannin was only detected in the leaf extract of *A. digitata* but was absent in the seed and pulp extracts. This agrees with the findings of Ayele et al. (2013), who determined the total polyphenol content of *A. digitata* leaves and expressed it as tannic acid equivalents. Previous work has also proven tannins to be present in *A. digitata* bark (Tanko et al. 2008). The stem bark of *A. digitata* is known to contain both soluble and insoluble tannins (Yusha'u et al. 2010).

Saponin was present in both the pulp and leaf extract of *A. digitata,* but was not detected in its seed extracts. Warra et al. (2012), however, reported slight presence of saponin in *A.digitata* seed oil, but this could be attributed to the solvent used in extraction, which was hexane. The fruit pulp of *A. digitata* has been found to have

anti-inflammatory properties; this is often attributed to the presence of sterols, saponins and triterpenes in its aqueous extract (Ramadan et al. 1994).

Terpenoid was present only in the pulp extract of *A. digitata* but not in its seed and leaf extracts. This is also consistent with the findings of Al-Qarawi et al. (2003), who reported isolating triterpene from *A. digitata* fruit. In research carried out by Warra et al. (2012) though, terpenoids were found to be present in an extract of *A. digitata* seeds. This might be because the seeds were extracted with a different solvent, namely hexane.

Alkaloids, flavonoids, phenol, tannins, terpenoids and saponins were present in the bark extracts of *G. livingstonei* and *S. birrea,* with the exception of the water extract of *S. birrea,* which did not contain any flavonoid. Phytochemistry of the stem bark of *S. birrea* in previous work has been reported to contain tannins, flavonoids, alkaloids, and steroids (Ojewole et al. 2010). Previous work also showed that xanthones and biflavonoids had been isolated from the root bark of *G. livingstonei* (Mbwambo et al. 2006), while benzophenones and biflavonoids were isolated from its fruit (Yang et al. 2010). The significance of this finding is that bark extracts of *G. livingstonei* and *S. birrea* are a good source of alkaloids, flavonoids, phenol, tannins, terpenoids and saponins.

Tannins and flavonoids are known for their laxative effect and are thought to be responsible for antidiarrhoeal activity by increasing colonic water and electrolyte reabsorption (Palombo 2006; Misra et al. 2014), which explains why the bark of these plants are used traditionally in treating diarrhoea.

Terpenoids have been shown to be active against bacteria, fungi, viruses and protozoa and their mechanism of action is speculated to involve membrane

disruption by the lipophilic compounds (Cowan 1999). Alkaloids have been found to have antimicrobial properties with microbicide effects against *Giardia* and *Entamoeba* species, as well as antidiarrheal effects which are probably due to their effects on transit time in the small intestine (Cowan 1999). Saponins have several biological effects, some of which are antibacterial, antifungal, antiparasitic, antitumours/cytotoxicity, antiviral and antioxidant activities (Sparg et al. 2004).

The serial dilution done on a 96-well microtitre plate for the antioxidant assay did not give a 50% inhibition for DPPH when the absorbencies were read on the ELISA plate reader. Further dilution from the last well of the initial plate had to be done in a new microtitre plate. The absorbencies of the second plate were read on the ELISA machine and 50% inhibition was observed.

Water and methanol extracts of bark of *S. birrea* and *G. livingstonei* exhibited excellent antioxidant activities with their 50% inhibitory concentration of DPPH radical ranging from 0.28±0.02 µg/ml to 0.40±0.02 µg/ml. This is quite impressive when compared to the positive control, vitamin C, which had a 50% inhibitory concentration of 10.62±0.87 µg/ml. These results can be attributed to the presence of phenols, flavonoids, tannins, alkaloids, saponins and terpenoids and this is in agreement with studies in which these compounds have been associated with high antioxidant activities (Dangles et al. 2000; Moura et al. 2007; Gulcin et al. 2004; Grabmann 2005).

Extracts of *A. digitata* leaves also exhibited good antioxidant properties, with their IC_{50} values being 2.79±0.07 µg/ml and 2.82±0.05 µg/ml. This can also be attributed to the presence of phenols, flavonoids, tannins and saponins.

CHAPTER 5: GENERAL DISCUSSION AND CONCLUSION

5.1 GENERAL DISCUSSION

Water and methanol extracts of *A. digitata* seeds and pulp showed no antimicrobial activity with both the agar well diffusion method and the broth microdilution method. Their phytochemical analysis, however, revealed that both plant parts contain flavonoids and phenols, while the extracts of *A. digitata* pulp also contain terpenoids and saponins. These phytochemicals might have contributed to the antioxidant properties exhibited by both water and methanol extracts of *A. digitata* seeds and pulp, though their methanol extracts exhibited better antioxidant properties than their water extracts. This is because methanol extracts more polyphenols such as flavonoids, which are thought to have antioxidant properties, better than water (Medini et al. 2014; Pietta 2000).

Water extract of *A. digitata* leaves showed lower antimicrobial activity when compared to its methanol extract. The MIC of the methanol extract for *A. digitata* leaves was lower than its water extract. This is because the methanol extract dissolved better in 10% DMSO than the water extract. Phytochemistry of the extracts of *A. digitata* leaves revealed that they contained flavonoid, phenol, tannin and saponin, which explains why the extracts of *A. digitata* leaves also had good antioxidant properties, as their IC_{50} ranged between 2.79±0.07 to 2.82±0.05 µg/ml. The presence of these phytochemicals also explains why the methanol extract in particular was effective against bacteria causing diarrhoea, as flavonoids, phenols, saponins and tannins have been implicated as antimicrobials (Cowan 1999; Sparg et al. 2004; Palombo 2006).

The result of the agar well diffusion method showed that the water and methanol extracts of *G. livingstonei* and *S. birrea* had antimicrobial potential, as they showed zones of inhibitions similar to the positive control ciprofloxacin. The broth microdilution method however, was more conclusive, as it indicated the concentrations at which these plant extracts showed inhibition of the test organisms. Similar values are seen amongst the MICs of extracts of *G. livingstonei* and *S. birrea*, ranging between 0.39 mg/ml to 1.56mg/ml. This may be attributed to the fact that the solvents used are both polar solvents and at such they extract similar antibacterial compounds and this justifies their traditional use in the treatment of diarrhoea and the method of preparation, which is usually infusion or decoction.

The phytochemical analysis of *G. livingstonei* and *S. birrea* extracts reveal that they all contain alkaloids, phenols, flavonoids, tannins, terpenoids and saponins, with the exception of the water extract of *S. birrea,* which did not contain any flavonoid. The IC_{50} of *G. livingstonei* and *S. birrea* extracts, which ranged from 0.28±0.02 to 0.40±0.02 µg/ml, is an indication that they were very good antioxidants, as vitamin C, which was the positive control, only had an IC_{50} of 10.62±0.87 µg/ml. Cytotoxicity assay also revealed the safety profile of *G. livingstonei* and *S. birrea* extracts. Their EC_{50} ranged from 105.9 µg/ml ±19.50 to 769.9 µg/ml ± 36.33 in comparison to Actinomycin D, which had an EC_{50} of 0.003 µg/ml ±0.000095. This shows that these plants extracts are tolerated by the human kidney cells even at high concentrations.

5.2 CONCLUSION

This study investigated the antimicrobial, antioxidant and phytochemical content of all the plant extracts, as well as the cytotoxicity of extracts of bark of *G. livingstonei* and *S. birrea.*

This study demonstrates that the water and methanol extract of *S. birrea,* and *G. livingstonei* were active against all the test organisms. They exhibited antioxidant activities and six phytochemical compounds were detected in them with the exception of the water extract of *S. birrea,* which contained five, as well as having a safe cytotoxicity profile.

The water and methanol extract of *A. digitata* leaves also exhibited inhibitory activities against the entire test organisms, had good antioxidant activities and four phytochemical compounds were detected in them.

The water and methanol extracts of *A. digitata* seeds and pulp, however, showed no antimicrobial activities but exhibited antioxidant properties and two phytochemical compounds were detected in *A. digitata* seed extracts, while four phytochemical compounds were found in the pulp extracts.

5.3 RECOMMENDATIONS

The agar well diffusion assay was not a conclusive method to determine the antimicrobial content of the plant extracts. A further conclusive test was therefore carried out using the broth microdilution method to determine the MICs and bactericidal concentrations of the plant extracts.

Further research needs to be carried out on the bark extracts of *G. livingstonei* and *S. birrea* to isolate and identify the active compound(s) responsible for their antimicrobial, antioxidant and low cytotoxic properties.

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